Original Article

Fertility Preservation Before Breast Cancer Treatment Appears Unlikely to Affect Disease-Free Survival at a Median Follow-Up of 43 Months After Fertility-Preservation Consultation

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BACKGROUND: The objective of this study was to determine whether fertility preservation (FP) with oocyte/embryo cryopreservation is associated with differences in disease-free survival (DFS). **METHODS:** This retrospective study included patients aged 18 to 45 who were diagnosed with invasive breast cancer between 2007 and 2017 and were seen for FP consultation at a university fertility center before cancer treatment. The primary endpoint, DFS, was defined as the time from FP consultation until patients developed a locoregional recurrence, distant metastasis, a contralateral breast tumor, or a new primary malignancy. DFS was compared for FP versus no FP using Kaplan-Meier survival estimates and Cox proportional-hazard regression analysis. **RESULTS:** The study included 329 women, with 207 (63%) in the FP group and 122 (37%) in the no FP group. Patients who underwent FP had more aggressive initial disease profiles than those in the no FP group. In addition, they were younger (35 vs 37 years; $P = .009$), more often had stage II or III disease (67% vs 55%; $P = .03$), and had higher rates of requiring chemotherapy (77% vs 65%; $P = .01$). Over a median follow-up of 43 months, the rates of DFS were similar among patients in the FP group and the no FP group (93% vs 94%, respectively; hazard ratio [HR] 0.7; 95% CI, 0.3-1.7). Positive ER status (79% vs 83%; $P = .38$), neoadjuvant chemotherapy (41% vs 48%; $P = .32$), ER-positive DFS (HR, 0.4; 95% CI, 0.1-1.6), and neoadjuvant chemotherapy DFS (HR, 1.4; 95% CI, 0.2-9.1) were similar in the FP and no FP groups, respectively. **CONCLUSIONS:** At a median follow-up of 43 months, FP appears unlikely to affect DFS, even in the setting of tumors with positive ER status or treatment with neoadjuvant chemotherapy (in which the tumor remains in situ during FP). ***Cancer* 2020;126:487-495**. © *2019 American Cancer Society*.

KEYWORDS: breast cancer, embryo cryopreservation, fertility preservation, oocyte cryopreservation, safety.

INTRODUCTION

Infertility as a result of cancer treatment can have a profound impact on the quality of life of reproductive-age survivors.[1] Fertility preservation (FP) techniques like oocyte or embryo cryopreservation have been associated with improved long-term quality of life.[2] Although the benefits of FP are becoming increasingly accepted, the safety of FP for women with breast cancer could be more fully explored. The ovarian stimulation process for oocyte/embryo cryopreservation takes about 14 days, and serum estradiol levels may reach levels from 10 to 20 times higher than those achieved during the natural menstrual cycle.[3] Because of concern about the mitogenic activity of estrogen toward breast cancer cells, medications such as tamoxifen or aromatase inhibitors are often used during ovarian stimulation for FP.[4,5]

FP for women diagnosed with breast cancer has been widely used for nearly 2 decades.[3,5-7] Historically, the safety of FP with breast cancer has been supported inferentially. Estrogen levels in pregnancy have been noted to be 100-fold higher than in the normal menstrual cycle and, consequently, from 5-fold to 10-fold higher than during ovarian stimulation. The levels of estrogen are also elevated over many months, whereas, in ovarian stimulation, they are elevated during approximately 7 of 14 days of ovarian stimulation. Thus, the observation that pregnancy after treatment for breast cancer appears to be safe has been used to support the safety of FP.[8,9]

Recently, the safety of ovarian stimulation in the setting of breast cancer has been more directly evaluated. Six studies, encompassing a total of 477 women who underwent ovarian stimulation, reported similar rates of breast cancer recurrence and mortality after FP versus no FP.[5,7,10-12] Although these studies have contributed very important data, they are limited by their observational nature, small patient numbers, lack of information about breast tumor and treatment type, older ovarian stimulation techniques, and relatively short duration of follow-up.[13] It is unlikely that there will be higher

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quality safety data from a randomized controlled trial of FP versus no FP in the future. Ethical and practical concerns would impede such a study: one cannot likely randomize someone to protect their ability to have a family or not.[13] Therefore, there is a need for more observational data, particularly from larger studies with modern ovarian stimulation and breast cancer treatment approaches. For example, a large study focused on the safety of FP in the setting of neoadjuvant chemotherapy has not yet been published. Neoadjuvant chemotherapy is becoming more widely use and is especially interesting to study for FP safety because the breast tumor remains in situ during FP.[14,15]

The objective of the current study was to determine whether FP is associated with differences in disease-free survival (DFS), particularly among women who undergo neoadjuvant chemotherapy for breast cancer.

MATERIALS AND METHODS

We performed a retrospective study. Informed written consent was obtained as part of a longitudinal survey study. All study procedures were approved by the University of California, San Francisco (UCSF) Committee on Human Research.

Study Population

Patients included in this study had been diagnosed with breast cancer and had been referred for discussion of FP options at the time of their initial cancer diagnosis. They were referred to our Reproductive Endocrinology Clinic at UCSF from oncology clinics around Northern California, both community-based and academic. Inclusion criteria for this retrospective study included: premenopausal state at the time of diagnosis, age 18 to 45 years, newly diagnosed with nonmetastatic breast cancer, and presenting for initial FP consultation visit. Patients were excluded for: age outside the range above, metastatic disease at initial presentation, lack of staging information at initial presentation, or receipt of chemotherapy or radiation treatment before FP consultation. The majority of oncology clinics in Northern California communicate with UCSF medical records through EPIC (EPIC Systems). Only patients who were seen at UCSF or at clinics whose computer systems communicated with UCSF EPIC were included in the study to ensure more adequate follow-up. Each woman was seen for FP consultation shortly after diagnosis and before chemotherapy (if any) for breast cancer during the years from 2007 to 2017. DFS (as defined below) was evaluated using chart review of routine clinical follow-up and with a longitudinal survey. If discrepancies were noted between the survey and clinical notes, then clinical documentation was considered to be most accurate.

Fertility Preservation

Patients were referred by their oncology team for FP consultation after a new breast cancer diagnosis. The reproductive endocrinology team discussed anticipated risks of infertility after cancer treatment and then offered FP with oocyte or embryo cryopreservation. With few exceptions, egg or embryo cryopreservation was offered to each patient, including those with advanced disease. Although we did routinely discuss the use of gonadotropin-releasing hormone agonists (GnRHa) as having a potential benefit for future gonadal function, we defined having undergone FP as cryopreserving oocytes or embryos. FP was defined as such because oocyte and embryo cryopreservation are accepted as standard treatment for FP by the American Society of Clinical Oncology, whereas medical treatment with GnRHa is not yet considered a proven FP method.[16]

The patients who did not pursue FP (oocyte or embryo cryopreservation) resumed care with their oncology team. Those who did pursue FP began ovarian stimulation as soon as possible after the initial FP consultation. Random-start ovarian stimulation was used whenever possible, as it allows for the collection of oocytes within 2 weeks of the initial FP consultation, in order to not further delay cancer-directed therapy.[17] Antagonist-based ovarian stimulation protocols were used. Ovarian stimulation was undertaken with recombinant follicle-stimulating hormone at doses from 75 to 300 international units (IU) in combination with human menopausal gonadotropins at doses from 75 to 150 IU.

Patients with ER-positive breast cancer who underwent FP were co-treated with letrozole or tamoxifen during the ovarian stimulation process. Letrozole has historically been used in our clinic for this application. Oral letrozole 5 mg daily was started with the first day of gonadotropins, titrated during the follicular phase of the cycle to keep estrogen levels below 500 pg/mL, and then continued through the end of the luteal phase. Oral tamoxifen 20 mg daily was used in place of letrozole for patient convenience (ie, she already knew she would take tamoxifen in an adjuvant setting) or when patients were randomized to tamoxifen as part of an ongoing clinical trial about the use of concomitant letrozole or tamoxifen during ovarian stimulation for women with breast cancer (the Fertility Preservation Using Tamoxifen and Letrozole in Estrogen Sensitive Tumors Trial [TALES]; clinicaltrials.gov identifier NCT03011684). Trigger with

human chorionic gonadotropin was used as a standard. When the risk of ovarian hyperstimulation syndrome was considered elevated, leuprolide and human chorionic gonadotropin combination triggers were used. Trigger was performed when at least 2 follicles reached a mean diameter of 18 mm in tamoxifen cycles and 20 mm in letrozole cycles, as supported by prior studies.[9] Once oocyte retrieval was complete, patients who underwent FP returned to their oncology care as soon as possible.

Follow-Up for DFS

DFS, our primary endpoint, was calculated in months from FP consultation until the patient developed a locoregional recurrence, distant metastasis, a contralateral breast tumor, or a new primary malignancy. The last date of follow-up was defined as the date of the most recent survey or the date of the most recent physician visit in the medical record. If there was no evidence of locoregional recurrence, distant metastasis, contralateral breast tumor, or new primary malignancy in the survey or the medical record, then the patient was deemed to be disease-free until that point in time, and data beyond that point were censored. For follow-up, the following were used: clinic visits, pathology reports, cancer treatment reports, surveys, and electronic medical record review.

Potential Confounders

Demographic characteristics, reproductive health history, cancer treatment, and tumor characteristics were recorded and de-identified. Patient age was recorded as age at the time of FP consultation. The following tumor characteristics were recorded: primary tumor size (≤20 mm, 21-50 mm, or >50 mm), lymph node positivity (yes or no), cancer stage (clinical staging for neoadjuvant chemotherapy and surgical pathology staging for adjuvant chemotherapy), *BRCA* positivity (yes or no), ER positivity (yes or no), and HER2-*neu* receptor positivity (yes or no). Cancer treatment characteristics that were recorded included: chemotherapy (yes or no), chest wall radiation (yes or no), chemotherapy timing (adjuvant or neoadjuvant), and type of adjuvant endocrine therapy (tamoxifen or aromatase inhibitors with surgical or pharmacologic ovarian suppression). Those potential confounders with P values <.2 between the FP and no FP groups were included in the multivariable survival analysis described below.

Statistical Analysis

Statistical analyses were performed using Stata version 15 (Stata Corporation). Two-sided P values <.05 were considered significant.

To attempt to control for potential biases in who decided to undergo FP versus who did not, particularly given the variable treatments of the multipractice referral network, we initially compared baseline characteristics among those who did and did not undergo FP. These comparisons were performed using Fisher exact tests, chi-square tests, t tests, or Wilcoxon rank-sum tests, as appropriate.

DFS analyses were carried out using Kaplan-Meier survival estimates and Cox proportional-hazard regression analysis. Kaplan-Meier DFS estimates were plotted for the FP and no FP groups. A power calculation was performed in Stata to assess the detectable hazard difference in the experimental (FP) and control (no FP) groups in our study. The following parameters were used for the calculation: 1) a maximum follow-up of 131 months, 2) a median follow-up of 43 months, 3) an estimated 92% survival rate in the control group at 43 months (based on data from Demicheli et al in premenopausal women[18]), and 4) 122 women in the control group and 207 in the experimental group. Our study was powered to detect a difference in hazards of 0.26 from the control to the experimental group. DFS was calculated for FP versus no FP using univariable Cox regression. Next, to assess the impact of different baseline patient factors on DFS for the entire FP versus no FP groups, multivariable Cox regression analyses were performed to adjust for the effect of potential confounding variables. Potential confounding variables were defined as those variables in the assessment of baseline differences between the FP and no FP groups for which the P value was <.2. Finally, multivariable Cox regression was performed, including the potential confounding variables mentioned above, for: 1) women with ER-positive tumors who underwent FP versus no FP and 2) women who received neoadjuvant chemotherapy (with their tumor in situ) and underwent FP versus no FP.

RESULTS

Study Population

Three hundred twenty-nine patients with breast cancer met the inclusion criteria and were included in the study (Fig. 1). In total, 207 women (63%) underwent egg or embryo cryopreservation before cancer treatment (FP), and 122 (37%) did not (no FP). Cancer follow-up information was available for a median follow-up of 43 months (range, 2-131 months). There was a trend toward slightly shorter follow-up in the FP group compared with the no FP group (median, 42 vs 46 months; $P = .09$).

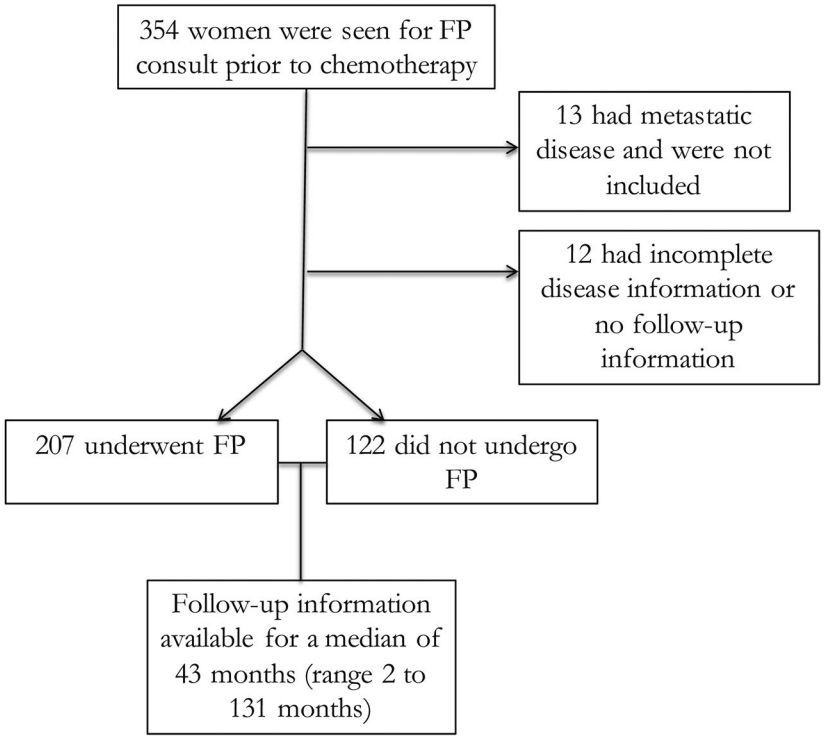

Figure 1. The total study population and follow-up rates are illustrated. In total, 354 women were seen for fertility-preservation (FP) consultation during the study period (2007-2017), and 329 women who had stage I to III breast cancer were included in the study. Two-thirds underwent FP, and one-third did not.

Baseline Characteristics: FP Versus No FP

The baseline characteristics of patients who underwent FP were suggestive of more aggressive disease (Table 1). Ovarian stimulation characteristics of FP cycles are displayed in Table 2. FP patients were younger in mean age at diagnosis (35 ± 5 years vs 37 ± 6 years; $P = .01$). Those who underwent FP were more likely to require chemotherapy (77% vs 65%; $P = .01$) and were more likely to have greater than stage I disease (66% vs 53%; $P = .02$) than those who did not undergo FP. There was also a trend toward more high-grade cancers (grade 3) in the FP group (47% vs 36%; $P = .053$).

Cancer treatment was otherwise similar between the 2 groups. Just under one-half of the women in the FP and no FP groups were treated for cancer at an academic medical center (42% vs 48%, respectively; $P = .26$). Similar numbers in the FP and no FP groups chose unilateral lumpectomy (as opposed to mastectomy; 47% lumpectomies in the FP group vs 51% in the no FP group; $P = .49$), and the percentage in the FP group versus the no FP group who elected for contralateral prophylactic mastectomies also was similar (27% vs 27%; $P = .96$). The percentage in the FP and no FP groups that received radiation was identical (65% vs 65%; $P = .99$). A similar percentage of women who underwent chemotherapy in the FP and no FP groups did so in the neoadjuvant setting (41% vs 48%, respectively; $P = .32$).

Although FP patients had more aggressive disease (eg, higher stage at diagnosis, younger age at diagnosis), overall, FP was not associated with a decrease in DFS. Over a median follow-up of 43 months, 22 patients developed a DFS endpoint: locoregional recurrence (n = 9), distant metastasis (n = 12), contralateral breast tumor (n = 1), or new primary malignancy (n = 0) (Table 3). Overall, Kaplan-Meier DFS estimates for the FP and no FP groups appeared to be similar (Fig. 2). Univariable Cox hazard ratios (HRs) for DFS during our median 43-month study period were similar among those undergoing FP versus no FP (93% vs 94%, respectively; HR, 0.7; 95% CI, 0.3-1.7) (Table 4). This relation remained similar, even after controlling for age at diagnosis, cancer stage, cancer grade, and whether or not chemotherapy was given (HR, 0.7; 95% CI, 0.2-2.0). The *P* value for tumor size was also <.2, but tumor size was omitted from the multivariable Cox regression model because it is collinear with cancer stage.

TABLE 1. Comparison of Patients Who Underwent Fertility Preservation Versus No Fertility Preservation

Variable	FP, n = 207	No FP, n = 122	*P*
Age at FP consult (y)	35 ± 5	37 ± 6	.01
Primary tumor size, %			
<20 mm	52	62	.19
21-50 mm	44	34	
>50 mm	5	4	
Lymph node-positive, %	43	38	.36
Stage, %			
I	34	47	.07
II	53	42	
III	11	10	
Tumor grade, %			
1	15	17	.15
2	38	47	
3	47	36	
BRCA-positive, %	12	10	.63
ER-positive, %	79	83	.38
HER2-positive, %	31	25	.28
Lumpectomy, %	47	51	.49
Bilateral mastectomy, %	27	27	.96
Received chemotherapy, %	77	65	.01
Neoadjuvant chemotherapy, %	41	48	.32
Received radiation therapy, %	65	65	.99
Cancer treatment in university hospital, %	42	48	.26
Adjuvant hormone therapy (among ER-positive), %			
Tamoxifen	85	85	.98
Letrozole plus ovarian suppression	15	15	

Abbreviations: ER, estrogen receptor; FP, fertility preservation; HER2, human epidermal growth factor receptor 2.

Outcome After Ovarian Stimulation With ER-Positive Disease or Tumor in Situ

After controlling for age at diagnosis, cancer stage, cancer grade, and chemotherapy exposure (yes/no), patients with ER-positive tumors who underwent FP had rates of DFS similar to those who did not undergo FP (DFS: HR, 0.4; 95% CI, 0.1-1.6). Those who underwent FP in the setting of neoadjuvant chemotherapy had similar rates of DFS to those who did not after adjustment for age at diagnosis, cancer stage, and cancer grade (DFS: HR, 1.4; 95% CI, 0.2-9.1).

DISCUSSION

This is the largest study to date from a single FP center to examine the safety of ovarian stimulation in the setting of newly diagnosed breast cancer. With hundreds of patients and over a median follow-up of 43 months (range, 2-131 months), FP was not associated with decreased DFS. And it is important to note that FP did not appear to increase risk even among women with ER-positive tumors and among those who underwent FP before neoadjuvant chemotherapy, while their tumor remained in situ.

TABLE 2. Ovarian Stimulation Cycle Characteristics[a]

Ovarian Stimulation Cycle Characteristics	Average Outcome ± SD
Antral follicle count	14 ± 9
Duration of ovarian stimulation, d	12 ± 2
Total dose of gonadotropins, IU	2522 ± 975
Peak estradiol during stimulation, ER-negative tumors, pg/mL	2538 ± 1746
Peak estradiol during stimulation, ER-positive tumors, treated with letrozole during ovarian stimulation, pg/mL	692 ± 401
Peak estradiol during stimulation, ER-positive tumors, treated with tamoxifen during ovarian stimulation, pg/mL	3018 ± 2016
Follicles >10 mm at time of oocyte collection	17 ± 11
No. of oocytes collected	19 ± 12
Mature oocytes, %	86
2PN fertilized/2PN mature oocyte, %	76

Abbreviations: 2PN, 2 pronuclei (ie, normally fertilized); IU, international units.
[a]In total, 116 women froze eggs, and 109 created embryos. Data are listed as the mean ± SD unless otherwise indicated.

The results of this study appear likely to be generalizable. Young women are more likely to present with more advanced stages of breast cancer because of diagnostic delays and more aggressive pathology.[15,19] In our study, the majority of women who were seen for FP consult had stage II or higher disease. There were also relatively high proportions of women with HER2-positive and ER-negative histology in our study, proportions that were similar to those reported in other large studies that included women of reproductive age.[20,21]

The DFS event rates of 6% to 7% after FP and no FP in our study were similar to those seen in prior studies that examined recurrence after FP. Meirow et al followed 27 women who underwent FP over a period of 3 to10 years and noted a 6% rate of recurrence.[5] Ben-Haroush et al noted a recurrence rate of 8% among 23 women who underwent FP and who were followed for 20 to 52 months. Interestingly, 1 group noted a similar risk of recurrence (9%) without the use of estrogen-modulating medications.[11,12] Finally, Rodriguez-Wallberg and colleagues recently published a registry-based study of 378 women with newly diagnosed breast cancer over the past 20 years in which similar rates of recurrence also were seen among those who did and did not undergo FP. In that study, 188 patients underwent FP, although 21% of these underwent FP without ovarian stimulation (natural cycle in vitro fertilization).[22]

Prior studies of the safety of FP in the setting of newly diagnosed breast cancer did not evaluate the safety of FP in the neoadjuvant treatment setting, in which the tumor remains in situ during FP. In our study, nearly

TABLE 3. Events That Disrupted Disease-Free Survival for Fertility Preservation Versus No Fertility Preservation[a]

FP or No FP	Time From FP Consult to Event, mo	Original Greatest Tumor Dimension, mm	Original Tumor Location	Recurrence Location	Locoregional Recurrence	Contralateral New Primary	Distant Metastases
FP	13	5	Left breast	Left breast	Yes		
FP	13	33	Right breast	Liver and bone			Yes
FP	17	14	Right breast	Right axilla	Yes		
FP	21	<20	Left breast	Lungs, axillary lymph nodes			Yes
FP	27	57	Left breast	Lung, bone			Yes
FP	33	75	Right breast, lymph nodes	Liver, bone			Yes
FP	39	16	Left breast, lymph nodes	Lung			Yes
FP	40	80	Left breast	Liver, bone, axillary lymph nodes			Yes
FP	46	21	Right breast, lymph nodes	Liver, bone			Yes
FP	47	19	Left breast, lymph nodes	Liver			Yes
FP	53	15	Left breast	Right chest wall			Yes
FP	58	11	Left breast	Left breast, right breast		Yes	
FP	60	14	Left breast	Chest wall	Yes		
FP	63	25	Right breast, lymph nodes	Right breast	Yes		
FP	68	22	Right breast, lymph nodes	Bone			Yes
No FP	19	23	Right breast, lymph nodes	Subcutaneous tissue, right breast	Yes		
No FP	19	31	Left breast, lymph nodes	Lung			Yes
No FP	32	70	Left breast, lymph nodes	Lung, liver, bone			Yes
No FP	33	3	Left breast	Left breast	Yes		
No FP	38	11	Left breast	Left breast	Yes		
No FP	47	9	Right breast, lymph nodes	Right breast	Yes		
No FP	83	11	Right breast	Right breast	Yes		

Abbreviation: FP, fertility preservation.

[a]This table lists the 15 of 207 events (7%) and the 7 of 122 events (6%) that disrupted disease-free survival for the patients in the study who did and did not undergo FP, respectively.

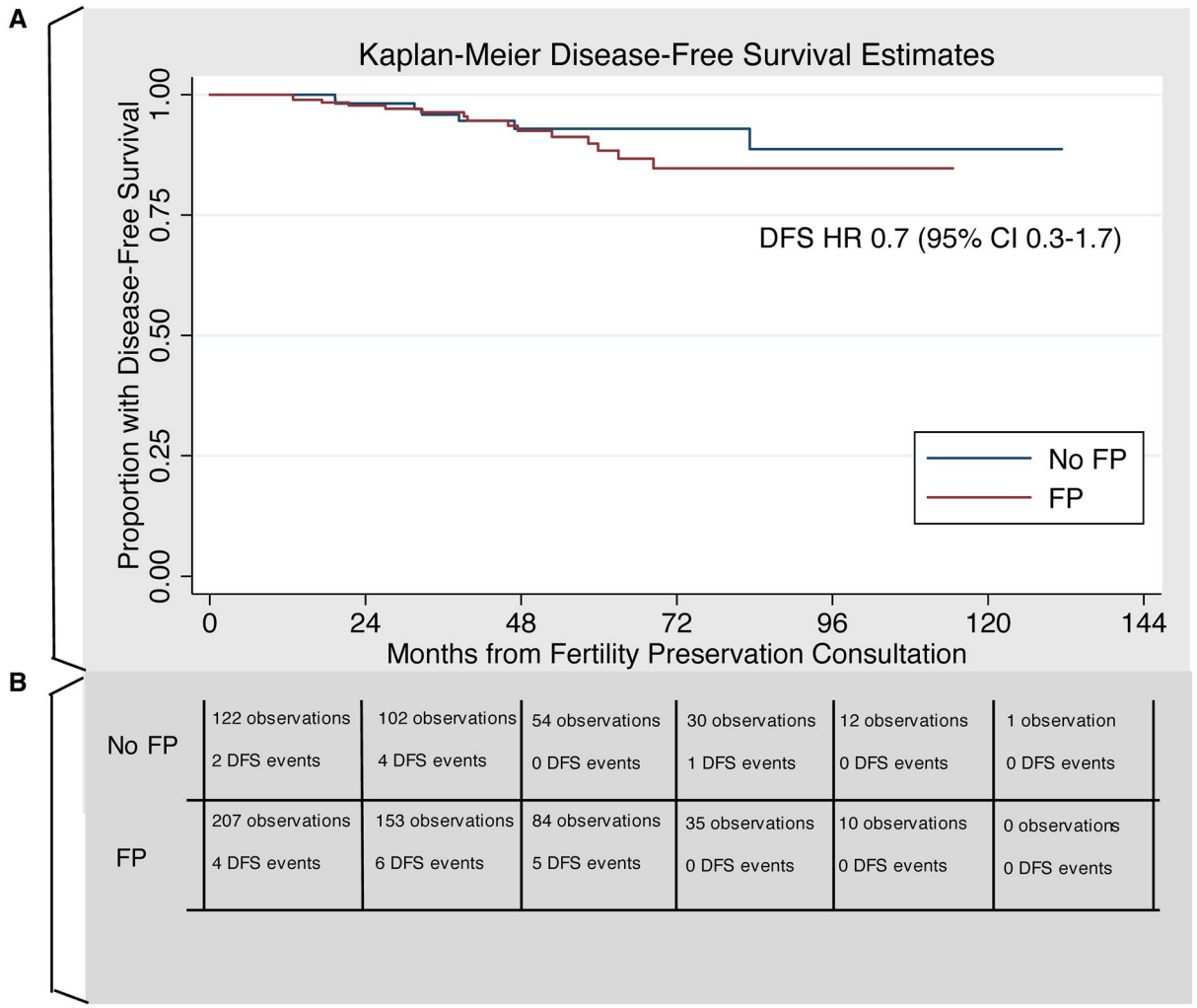

Figure 2. Kaplan-Meier disease-free survival (DFS) estimates are illustrated for all patients with invasive breast cancer who did and did not undergo fertility preservation (FP). (A) The pattern of DFS was similar over time, even after controlling for cancer stage, hormone receptor status, and treatment type (FP vs no FP; Cox proportional-hazard ratio [HR], 0.7; 95% CI, 0.3-1.7). (B) The number of patients who were followed for a minimum period is shown. For instance, 218 women in the FP group were followed for at least 0 to 24 months, and 31 women in the no FP group were followed for at least 72 to 96 months. DFS events (recurrences or mortalities) that occurred in a given period also are listed. For instance, 4 women had events in the first 0 to 24 months in the FP group, whereas 1 woman had DFS events in the period from 72 to 96 months in the no FP group. Most patients were followed for at least 43 months.

one-half of the patients underwent FP with a tumor in situ, and the DFS rates still were not changed, further supporting the finding that FP is unlikely to worsen breast cancer outcomes, particularly in the first several years after diagnosis. The levels of gonadotropins in our study were higher than those typically used in prior FP safety studies, supporting a likely lack of risk, even with dose escalation from natural cycle in vitro fertilization, to minimal ovarian stimulation with low-dose to high-dose gonadotropins (such as those used in our study).[5-7]

It is possible that data supporting the safety of FP could be confounded by women with more aggressive cancer being less likely to be advised to undergo FP. However, many of the prior FP safety studies did not display detailed baseline cancer characteristics. We have demonstrated that patients who chose to undergo FP were similar to those who did not in terms of disease and treatment type. With rare exceptions, we recommended consideration of FP (oocyte or embryo cryopreservation) to each patient. In fact, since those who underwent FP were 2 years younger at diagnosis and were more likely to have greater than stage I disease, one could argue that women who underwent FP had a slightly *more* aggressive pattern of disease at presentation.

TABLE 4. Overall Median Follow-Up and Disease-Free Survival: Fertility Preservation Versus No Fertility Preservation

Variable	FP, n = 207	No FP, n = 122	Statistical Output
Follow-up: Median (range), mo[a]	42 (2-114)	46 (10-131)	*P* = .09
DFS during study period, %	93	94	HR, 0.7 [95% CI. 0.3-1.7]

Abbreviations: DFS, disease-free survival; FP, fertility preservation; HR, hazard ratio.
[a]Over a median follow-up of nearly 4 years, the percentage of women who experienced DFS was similar among those who underwent FP and those who did not.

There are several strengths and limitations to our study. The study is relatively large in size and is limited to a single institution for fertility care. However, despite the relatively large size, the study is only powered to detect relatively large differences in DFS, so smaller differences could still exist and have not been detected by this study. In addition, heterogeneity did exist among the referring oncology clinics where referrals come from within our academic institution, as well as 4 other large community cancer centers. Although we did examine some of the most predictive factors for DFS in reproductive-age women, such as hormone receptor status, HER2 status, lymph node positivity, and chemotherapy (on a yes/no basis), there were several important factors that went unmeasured. For instance, we were unable to record the exact chemotherapy regimen and/or dose, as this information was not consistently available. We did capture BRCA status in most patients, but we did not calculate their Gail Model Risk Score or include information about other cancer-predisposition genes. Different chemotherapy regimens, including the combination of chemotherapeutics and whether or not the course is dose-dense, may have differing effects.[23,24] We also did not collect data to assess whether the patients in the neoadjuvant chemotherapy group had a complete or incomplete pathologic clinical response to their chemotherapy. There may have been differences in patient compliance with adjuvant endocrine therapy, which we did not measure in this study because it is difficult to capture.[19] Changing standards of antiestrogen treatments and duration of therapy in the ER-positive/PR-positive premenopausal breast population, which evolved nationally during our study period and were not directly measured in this study, may have been an unmeasured source of bias. We also did not assess referral bias because we do not know which reproductive-age patients in our referral network were not seen for FP consultation. However, as stated above, our study was similar on important disease metrics (lymph node status, receptors status, stage, etc) to those generally reported in the literature for reproductive-age women with breast cancer. We offered the option of medical treatment with concomitant GnRHa during chemotherapy. However, we did not have a reliable means of following which patients in the FP and no FP groups received GnRHa during chemotherapy. Thus, we defined FP by the American Society of Clinical Oncology standard of accepted treatment types, including oocyte or embryo cryopreservation. Finally, an important strength of our study is that we had similar follow-up time in both of our groups, minimizing the chance of detection bias affecting results.

Our study demonstrates that it is unlikely that FP increases cancer progression in the first several years after treatment. The "Folkman effect" describes a "double-hump" or typical bimodal pattern of breast cancer recurrence, with a first peak at about 1 to 2 years. The first peak may be because of increased angiogenesis to the tumor bed after surgery. A second peak typically starts around 5 years after breast cancer surgery and can last out to 15 to 20 years. The second peak may be because of the natural course of tumor growth.[25,26] Premenopausal patients have been shown to have an initial mortality wave covering about 6 years, with maximum height at the fourth year, followed by a peak 8 years after surgery, whereas postmenopausal patients showed an early high-mortality surge peaking at the third year, followed by a modest increase at the eighth year.[18] Although most high-risk recurrences in reproductive-age women occur during the time period covered by our study, it is possible that a delayed second peak could present and that this peak may depend on a different set of biologic circumstances than the first. We look forward to the results of other long-term follow-up studies, including the PREFER (PREgnancy and FERtility) study by Lambertini et al, in which patients with newly diagnosed breast cancer will be followed for up to 15 years after FP.[27]

Conclusion

Over a median follow-up of 43 months, FP with egg or embryo cryopreservation appears unlikely to be associated with a difference in DFS among women with breast cancer. Even among women who undergo FP in the neoadjuvant treatment setting, in which the tumor remains intact during FP, FP appears likely to be safe. This report adds to an important body of observational data, but more data points and longer periods of observation are warranted.

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CONFLICT OF INTEREST DISCLOSURES
The authors made no disclosures.

AUTHOR CONTRIBUTIONS
Joseph M. Letourneau had an equal role in conceptualization, methodology, formal analysis, investigation, and writing/editing the original draft and had a lead role in data curation and project administration. **Kaitlyn Wald** had a supporting role in data curation and writing/editing the original draft. **Nikita Sinha** had a supporting role in data curation and writing/editing the original draft. **Eve Harris** had a supporting role in writing/editing the original draft. **Flor Juarez-Hernandez** had a supporting role in data curation and writing/editing the original draft. **Marcelle I. Cedars** had a supporting role in conceptualization, formal data analysis, funding acquisition, and writing/editing the original draft. Charles E. McCulloch had a supporting role in conceptualization and writing/editing the original draft. **Milana Dolezal** had a supporting role in conceptualization, formal data analysis, and writing/editing the original draft. **A. Jo Chien** had a supporting role in conceptualization, formal data analysis, and writing/editing the original draft. **Mitchell P. Rosen** had an equal role in conceptualization, methodology, formal analysis, investigation, and writing/editing the original draft and also had a supporting role in data curation and funding acquisition.

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